

# WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: C12N 15/00, A01K 67/027, C07K 14/81,		(11) International Publication Number: WO 00/25578		
C12N 15/00, AUTK 0//02/, C0/K 14/61, C12N 5/06	A3	(43) International Publication Date: 11 May 2000 (11.05.00)		
(21) International Application Number: PCT/US9 (22) International Filing Date: 2 November 1999 (0	(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).			
(30) Priority Data:  Not furnished 2 November 1998 (02.11.98) 60/131,061 26 April 1999 (26.04.99) 60/131,328 26 April 1999 (26.04.99) 60/149,317 17 August 1999 (17.08.99)  (71) Applicant (for all designated States except US): TRI OF TUFTS COLLEGE [US/US]; Medford, MA 021  (72) Inventors; and (75) Inventors/Applicants (for US only): BAGUISI, Alexa [PH/US]; Nine Millbury Street, Grafton, MA 015 OVERSTROM, Eric, W. [US/US]; 32 Blanchar Grafton, MA 01519 (US).  (74) Agents: PIERCE, N., Scott et al.; Hamilton, Brook, Reynolds, P.C., Two Militia Drive, Lexington, M. (US).				
(54) Title: METHODS FOR CLONING ANIMALS	<del> </del>			

#### (57) Abstract

The present invention pertains to methods for cloning animals. In particular, the invention includes methods of cloning an animal by combining a genome from an activated donor cell with an activated enucleated oocyte to thereby obtain a nuclear transfer embryo, and impregnating an animal with the nuclear transfer embryo in conditions suitable for gestation of a cloned animal. The invention further relates to methods of chemically enucleating an oocyte having a meiotic spindle apparatus by exposing the oocyte with a compound that destabilizes the meiotic spindle apparatus.

## FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senega!
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico ·	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
СН	Switzerland	KG	Kyrgyzstan	NO	Norway	zw	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		
1							
1							

Interr nat Application No PCT/US 99/25786

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/00 A01K67/027 C07K14/81 C12N5/06 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) A61K A01K C07K C12N IPC 7 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ° Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. X. WO 99 01163 A (UNIV MASSACHUSETTS) 1-5,7-9, 14 January 1999 (1999-01-14) 11-17, 19-26, 28-30 page 11, line 23 -page 12, line 21 page 16, line 20 -page 19, line 7; claims 1-13, 15-22 CIBELLI J B ET AL: "CLONED TRANSGENIC 1-5,7 Α CALVES PRODUCED FROM NONQUIESCENT FETAL FIBROBLASTS" SCIENCE, US, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, vol. 280, 22 May 1998 (1998-05-22), pages 1256-1258, XP000786003 ISSN: 0036-8075 the whole document -/--Further documents are listed in the continuation of box C. Patent family members are listed in annex. Special categories of cited documents : "I later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the \*A\* document defining the general state of the art which is not considered to be of particular relevance invention "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention filing date cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another "Y" document of particular relevance; the claimed invention citation or other special reason (as specified) cannot be considered to involve an inventive step when the document is combined with one or more other such docu-"O" document referring to an oral disclosure, use, exhibition or other means ments, such combination being obvious to a person skilled \*P\* document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report **3** 1. 03. 00 24 August 2000 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo ni, Chambonnet, F Fax: (+31-70) 340-3016

2

Intern and Application No
PCT/US 99/25786

Citation of document, with indication, where appropriate, of the relevant passages  ROBL J M ET AL: "SOMATIC CELL NUCLEAR TRANSPLANTATION IN CATTLE"  ANNUAL MEETING SOC. STUDY REPROD,	Relevant to claim No.
TRANSPLANTATION IN CATTLE" ANNUAL MEETING SOC. STUDY REPROD,,	1
vol. 58, 8 August 1998 (1998-08-08), page 25 XP000791151	
the whole document WO 96 26268 A (GENZYME TRANSGENICS CORP) 29 August 1996 (1996-08-29)	1
BAGUISI A, ET AL.: "Production of goats by somatic cell nuclear transfer." NAT BIOTECHNOL, vol. 17, no. 5, May 1999 (1999-05), pages	1-4, 6-19, 21-30
page 457, column 1, paragraph 2 page 459, column 1, paragraph 3 -column 2, paragraph 4 page 460, column 1, paragraph 2	31
BORDIGNON V ET AL: "TELOPHASE ENUCLEATION: AN IMPROVED METHOD TO PREPARE RECIPIENT CYTOPLASTS FOR USE IN BOVINE NUCLEAR TRANSFER" MOLECULAR REPRODUCTION AND DEVELOPMENT,US,NEW YORK, NY, vol. 49, January 1998 (1998-01), pages 29-36, XP000910821 cited in the application the whole document	31
WO 98 29532 A (PEURA TEIJA ;UNIV MONASH (AU)) 9 July 1998 (1998-07-09) the whole document	31
WO 95 03398 A (UNIV MONASH ;TATHAM BRENDAN GEORGE (AU)) 2 February 1995 (1995-02-02) the whole document	31
US 5 952 222 A (ROSENKRANS JR CHARLES F ET AL) 14 September 1999 (1999-09-14) the whole document	31
	the whole document  BAGUISI A, ET AL.: "Production of goats by somatic cell nuclear transfer."  NAT BIOTECHNOL,  vol. 17, no. 5, May 1999 (1999-05), pages 456-461, XP000891364  page 457, column 1, paragraph 2  page 459, column 1, paragraph 3 -column 2, paragraph 4  page 460, column 1, paragraph 2  page 460, column 2, paragraph 1  BORDIGNON V ET AL: "TELOPHASE ENUCLEATION: AN IMPROVED METHOD TO PREPARE RECIPIENT CYTOPLASTS FOR USE IN BOVINE NUCLEAR TRANSFER"  MOLECULAR REPRODUCTION AND DEVELOPMENT, US, NEW YORK, NY, vol. 49, January 1998 (1998-01), pages 29-36, XP000910821 cited in the application the whole document  WO 98 29532 A (PEURA TEIJA ;UNIV MONASH (AU)) 9 July 1998 (1998-07-09) the whole document  WO 95 03398 A (UNIV MONASH ;TATHAM BRENDAN GEORGE (AU)) 2 February 1995 (1995-02-02) the whole document  US 5 952 222 A (ROSENKRANS JR CHARLES F ET AL) 14 September 1999 (1999-09-14)

...ternational application No. PCT/US 99/25786

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This international Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
As far as claims 8 to 30 are directed to a method of treatment of the non human animal body, the search has been carried out and based on the alleged effects of the composition.
Claims Nos.:     because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This international Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the dalms; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  X  No protest accompanied the payment of additional search fees.

# FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-30

Methods for cloning an animal or producing a transgenic animal comprising the steps of:

- a) combining a genome from an activated donor cell with an activated, enucleated oocyte to thereby form a nuclear transfer embryo; and:
- b) impregnating an animal with the nuclear transfer embryo in conditions suitable for gestation of the cloned animal.

#### 2. Claims: 31-38

A method of enucleating an oocyte having a meiotic spindle apparatus, comprising exposing the oocyte with at least one compound that destabilizes the meiotic spindle apparatus;

normation on patent family members

• ....

Inter Inal Application No PCT/US 99/25786

Patent document cited in search report		Publication date		atent family nember(s)	Publication date
WO 9901163	Α	14-01-1999	AU EP	8154398 A 1017422 A	25-01-1999 12-07-2000
WO 9626268	A	29-08-1996	US AU AU CA EP	5843705 A 695249 B 5026296 A 2197675 A 0773992 A	01-12-1998 13-08-1998 11-09-1996 29-08-1996 21-05-1997
WO 9829532	Α	09-07-1998	AU	7891298 A	31-07-1998
WO 9503398	A	02-02-1995	AU AU	687422 B 7260194 A	26-02-1998 20-02-1995
US 5952222	Α	14-09-1999	NONE	·	